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ULTRASTRUCTURE OF A CEPHALOPOD PHOTOPHORE. II. IRIDOPHORES AS REFLECTORS AND TRANSMITTERS

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Animals that live in the deep sea confront predation pressures concerned with background illumination (Denton, 1970). When viewed from below a pelagic animal at moderate depth casts a shadow which makes it clearly visible to any potential predator below it. In many fishes the problem is, in part, solved by the evolutions of highly reflective sides which reflect light efficiently enough so that it matches the background illumination except when viewed from directly below. This latter problem is frequently diminished either by the fish becoming laterally compressed so it presents a minimum outline when viewed from below, or by the development of bioluminescent countershading which duplicates the background illumination. A third potential solution, that of evolving transparent tissues, is only partially successful because some tissues must be opaque to function. For example the eyes must receive light from only one direction or, in the cephalopods, the ink sac by its very nature cannot be transparent.

Any organism which uses light as a countershading device has to match the physical characteristic of the background illumination. Daylight surrounding an animal swimming at a depth of a few hundred meters is limited in spectrum to the blue band because of the absorbtion characteristic of water. Furthermore, downwelling light is very directional and varies in intensity in relation to depth. For a bioluminescent countershading mechanism to be effective the photogenic organs must be able to simulate these light conditions. Recently Young (1972) has described mechanisms of countershading control involving feedback from extraocular organs of the cephalopods. This paper describes the iridophores of the photophores of *Pterygioteuthis microlampas* and offers speculation of how they might function in effective control of bioluminescent countershading.

Methods and Observations

The techniques used in this study were described in our previous paper (Arnold and Young, 1974). The light microscope observations on freshly dissected photophores were made on shipboard in a darkroom.

The iridophores in the photophore of *Pterygioteuthis microlampas* are of three major kinds with one additional kind occasionally encountered in the anterior capsule region. In addition, a reddish-brown pigment surrounds most of the posterior capsule and is evident even in 1 μ thick sections. Each of these iridophore types is individually described below and the brown pigment is briefly included for the sake of completeness. Based on their morphology and location, the iridophores can be given descriptive names. The anterior and posterior cap iridophores are

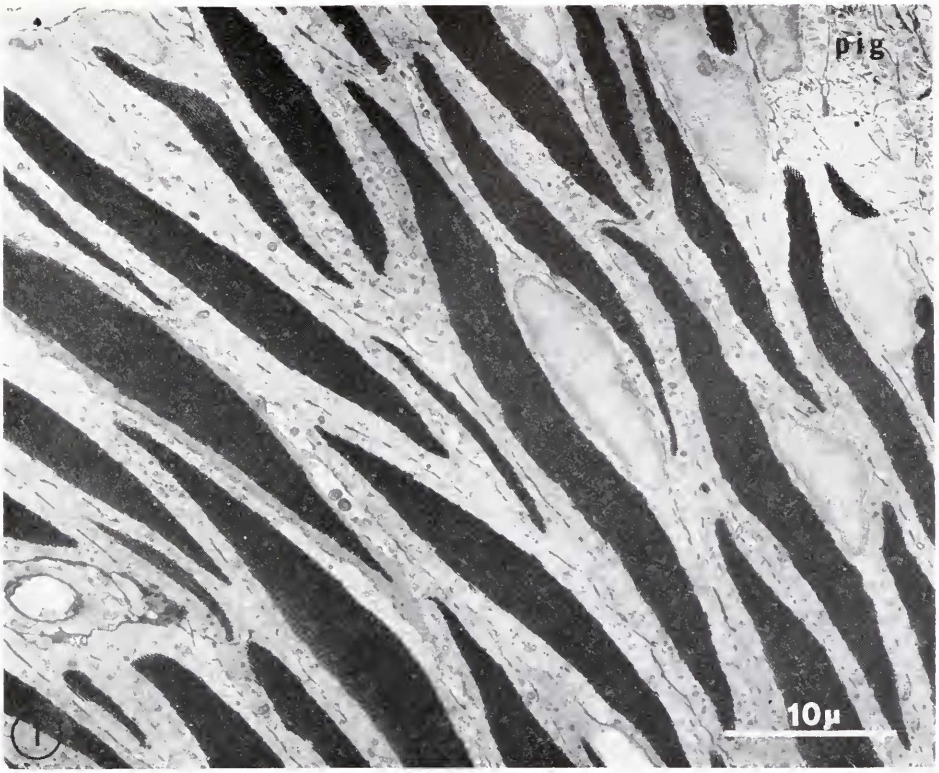
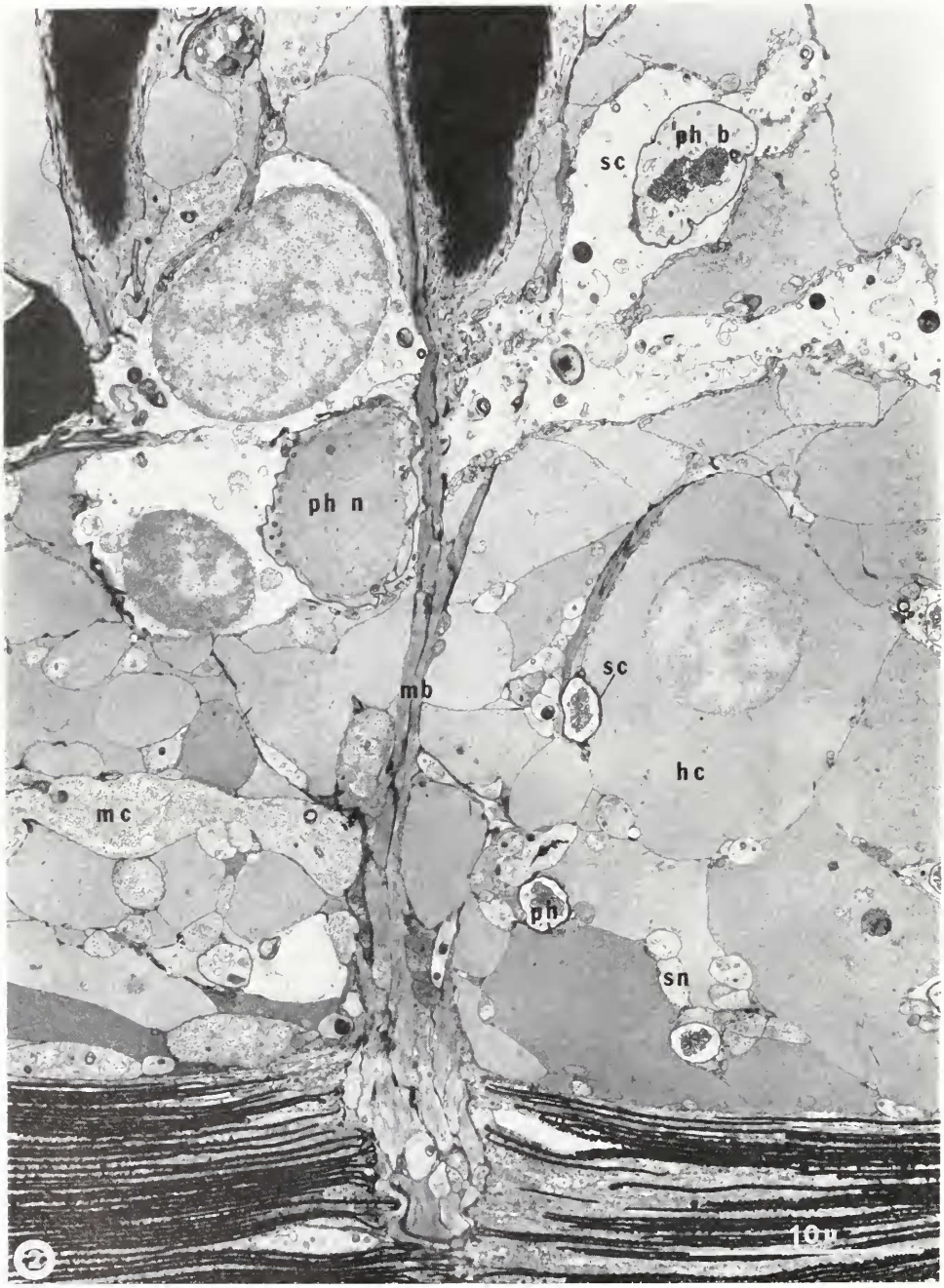


FIGURE 1. Posterior cap regular iridophores showing the single iridosome within each iridophore. The iridophores are separated by a discontinuous layer of intercellular substance. Some of the brown pigment spicules are evident in one corner (pig).

referred to here as "regular iridophores"; those of the inner funnel are called "irregular iridophores" (occasionally they also are found in the skin outside the photophore); and those in the clear lens are called "transparent iridophores" because they transmit light. The other terminology used here is that of Arnold (1967).

Regular iridophores

The regular iridophores are characterized by a highly ordered layering of the individual iridosomal platelets which alternate with extracellular spaces. The platelets are very constant in thickness as is the space between them. The platelets average 75 nm in thickness (range 69 nm–90 nm) and the space between platelets averages 53 nm (range 34 nm–66 nm). The platelets run straight for long distances and follow the general shape of the cell, being slightly curved in the posterior cup or wavy in some regions of the anterior cap. There is one iridosome per iridophore, and it occupies almost the entire volume of the cell (Fig. 1). When viewed with a dissecting microscope individual iridocytes can be teased out of the posterior cup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and appear like silvery mirrors which reflect rather than transmit light. Each regular iridophore is joined to its neighbors by an interrupted dense intracellular substance. The spindle-shape cells overlap to form a continuous layer in the posterior capsule or a thick lentoid block in the anterior capsule. In the center of the axial cone, individual iridophores are stacked more or less above one another. Each of the axial cone iridophores terminates with a muscle junction which continues across the axial cone through the inner funnel and attaches someplace outside the posterior capsule (Fig. 2). Similar strands of muscle also are seen between the iridophore and the rest of the anterior cap but not in the posterior cup region.

The platelets are remarkably parallel and maintain constant spacing over the entire length of the iridosome. At their ends the platelets seem to end in an oblique line rather than all at right angles to their collective long axes, hence the iridosomes are trapezoidal or lentoid in shape (Fig. 1). Each platelet is membrane bounded and these membranes appear to arise by fusion of anastomosing vesicles which originate in the cytoplasm of the iridophores and are continuous with the extracellular space as described in *Loligo* iridocytes (Arnold, 1967). The platelets themselves apparently are formed by the coalescence of dense granular material first into droplets which fuse to a discontinuous reticulum, then into a solid platelet (Figs. 3, 4). The iridosome thus formed is more or less centered in the cell and the nucleus is displaced to one side. The iridophores of the anterior cap are frequently wavy and appear somewhat compressed but still maintain the regular packing between the platelets and intraplatelet space. These iridophores, and in particular, those in the "plug" in the axial cone are separated by a prominent flocculent intercellular materal (Fig. 5). There is a slight but consistent thickness and spacing difference between the iridosomal platelets of the posterior cup and the anterior cap with the iridophores of the posterior cup having an average thickness of 69 nm (range 69 nm–59 nm) and spacing of 50 nm (range 57 nm–44 nm) while the anterior cap platelets average 85 nm (range 90 nm–78 nm) and are spaced at an average of 57 nm (range 50 nm–66 nm). With respect to their electron density, development, and other morphological respects, the iridophores of the anterior cap and the posterior cup appear to be similar.

Irregular iridophores

In contrast to the regular iridophores, the irregular iridophores are extremely variable in their shape, platelet thickness and arrangement, and interplatelet spacing (Fig. 6). They occur in the inner funnel of the photophore and are occasionally found in the skin outside (usually below) the photophore. The platelets are extremely variable in thickness but generally are thicker than the regulars (average

FIGURE 2. Muscle band (mb) traversing the axial cone and irregular iridophore layer. Note the muscle is attached to the regular iridophore of the "plug." The photogenic tissue is composed of several cell types but dominated by a homogeneous packing tissue (hc). Dendritic processes of the photocytes (ph) are surrounded with a dense sheath or a sheath cell (sc). A mitochondrial cell (mc) interdigitates between the packing cells. Within a sheath cell the nucleus (ph n) and one branch (ph b) of a developing photocyte can be seen. Several processes reminiscent of synapses are evident (sn).

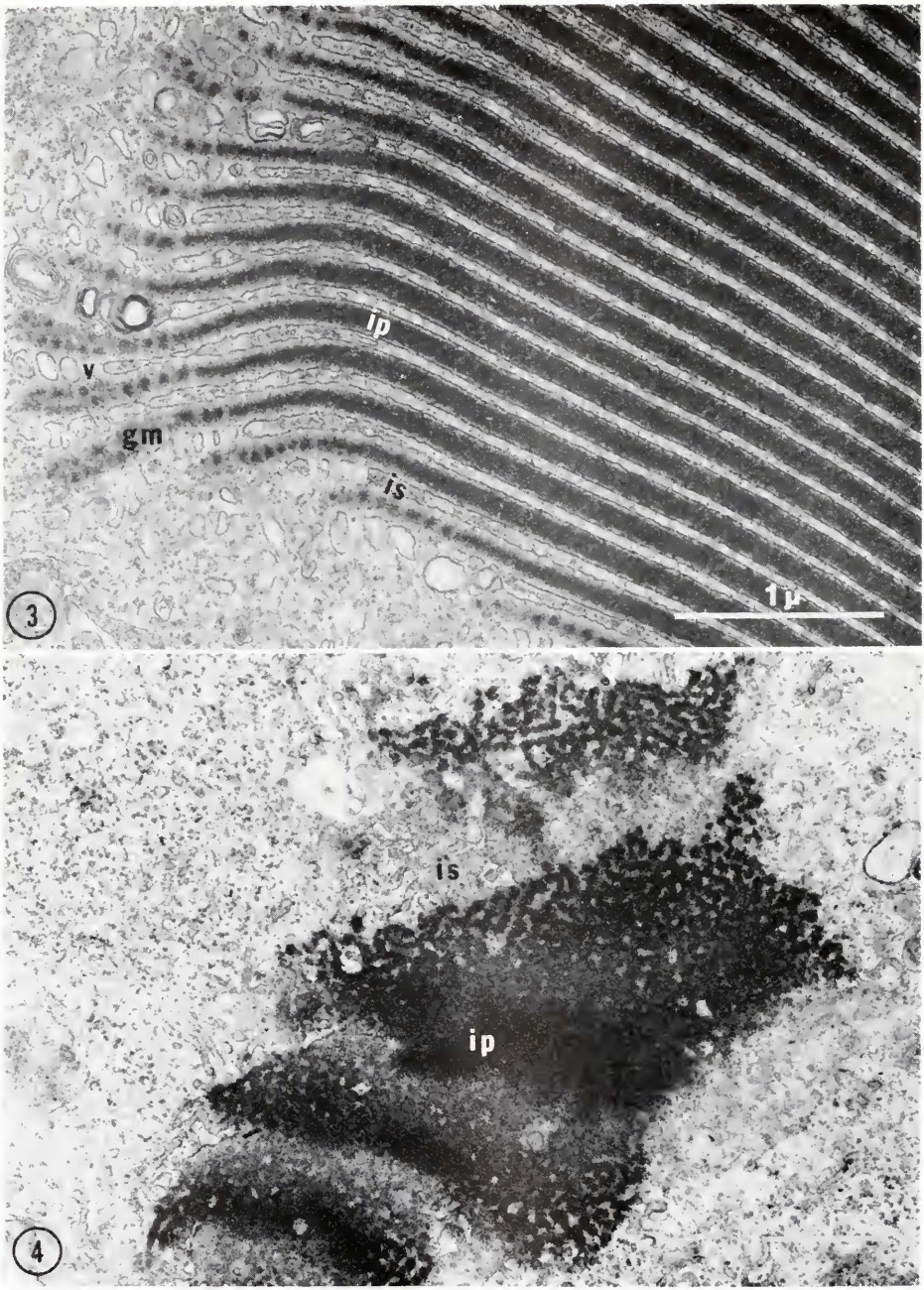


FIGURE 3. End of a regular iridosome showing the formation of the platelets (ip) by fusion of granular material (gm) and the origin of the interplatelet space (is) by fusion of vesicles (v) whose membranes persist as the platelet membrane.

125 nm; range 100 nm–140 nm) and unevenly spaced (average 103 nm; range 58 nm–173 nm). In general the platelets run parallel and in approximately straight lines, but they are covered with irregular knobs which sometimes are hollow or vary in density (Fig. 7). The platelets end in cytoplasmic extensions which themselves are extremely variable in thickness, orientation, and shape. Occasionally microtubules can be seen associated with the end of the platelets in a fashion similar to the development of *Loligo* iridophores (Arnold, 1967). These iridocytes interlock, overlap, and in general are not delineated from each other so that an exact determination of the boundaries of each cell can be made. In general they surround the axial cone to form another cone (inner funnel) which is much thicker at the distal end than the proximal. The general orientation of the platelets is somewhat perpendicular to the abutting concentric regular iridophores of the posterior cup and those of the distal anterior cap. The inner funnel is frequently traversed with muscle strands which connect to the innermost anterior cap iridophores (Fig. 2).

The platelets of the irregular iridophores appear to arise by fusion of granular material and interplatelet space seems continuous with a vesicular network in the cytoplasm of the iridocyte as well as the extracellular space (Fig. 6, 7).

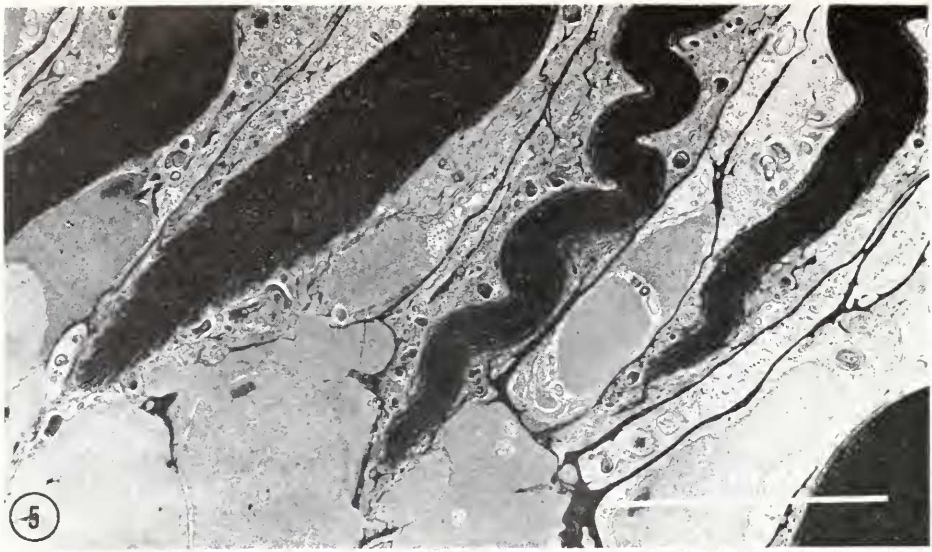
Transparent iridophores

Distal to the anterior cap of the photophore there is a transparent flexible layer which will be referred to here as the lens. The outer surface is covered by an epithelial layer with a highly convoluted surface, a dense basement membrane, and a layer of muscle fibers. The inner surface is underlaid by a continuous surface of pavement epithelium. The iridophores themselves are unique because they contain multiple iridosomes in which the platelets are in almost exact parallel register. In cross section each iridophore contains 20 to 50 such iridosomes arranged throughout the cytoplasm (Fig. 8). In section iridophores may contain as many as 51 platelets which probably represent the maximum number. The platelets average 43 nm in thickness (range 39 nm–45 nm) and are spaced at an average of 82 nm (range 73 nm–90 nm). The platelets are membrane bounded and the spaces between them are separated from the cytoplasm by a continuous plasma membrane. The platelets frequently have a discontinuous appearance and seem to be formed by coalescence of large droplets; although no obvious developmental stages of these iridosomes have been found. Most frequently the platelets seem to end in direct contact with the surrounding cytoplasm but there are examples of one end of an individual platelet being capped by a membrane while the other end is continuous with the cytoplasm (Fig. 9). Although it has not been possible to trace the membrane bounded space to a point continuous with extracellular space, by analogy from the regular and irregular iridophores it would seem that such continuity is likely.

Wide spaced iridophores

In addition to the three commonly encountered iridophores mentioned above, a fourth type of iridophore is infrequently encountered in the photophore or surround-

FIGURE 4. Glancing section of several iridophore platelets showing the reticulate nature of the platelets (ip) and the interplatelet space (is).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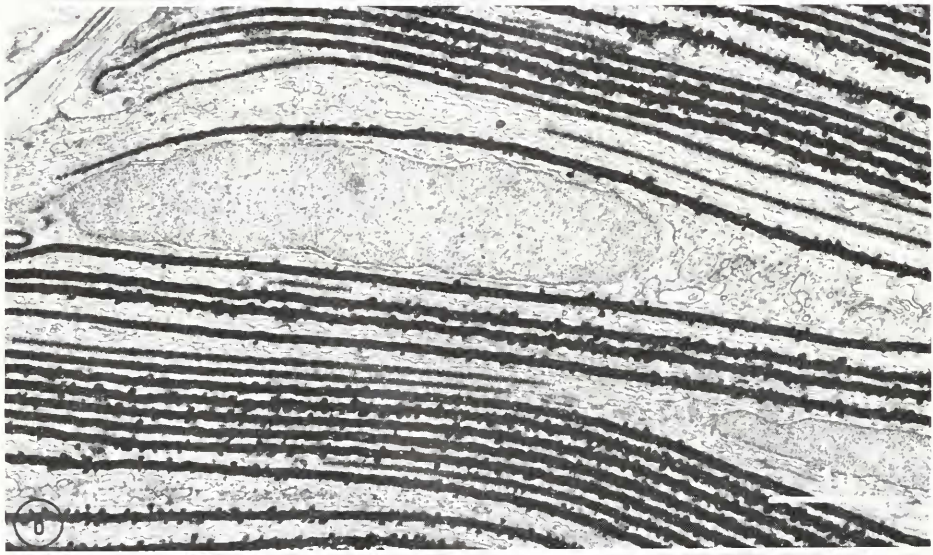
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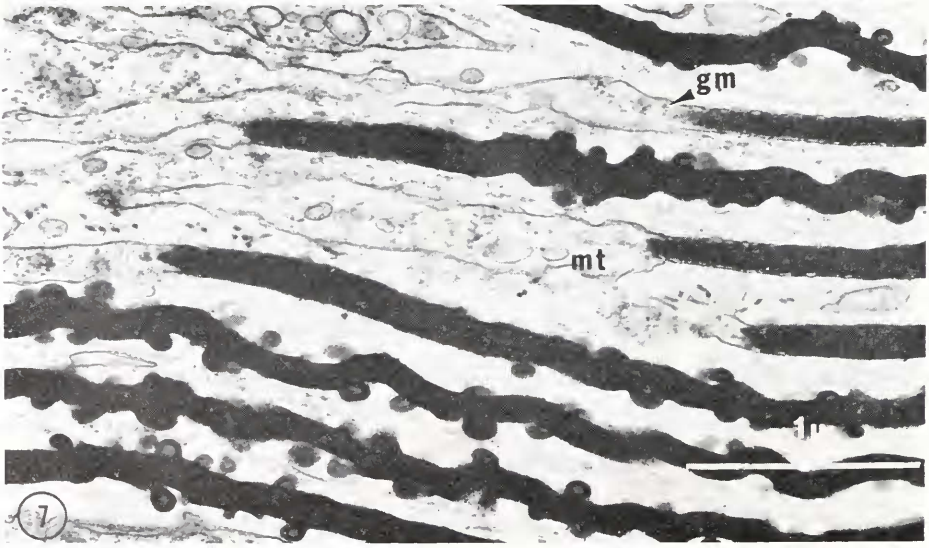

gm
mt
7

ing skin (Fig. 11). These iridophores are typified by wide irregular spacing (average 51 nm; range 42 nm–59 nm) and by platelets that follow a somewhat erratic alignment (average 84 nm; range 65 nm–120 nm. The single iridophore occupies a relatively small volume of the cell and the platelets appear to arise by fusion by dense granular material that becomes isolated between the fusing vesicles which form the interplatelet space (Fig. 11). These iridophores rarely occur between the transparent iridophores and the regular iridophores of the anterior cap and are occasionally encountered in the skin outside the photophore proper. Since they are so infrequently encountered, we have no data on their light reflecting characteristics.

Pigmentation of the capsule

In his description of photophores of *Pterygioteuthis,* Hoyle (1902 and 1904) mentioned a reddish-brown pigment layer which is found in the "connective tissue capsule" of the photophore. With the electron microscope, this pigment can be seen to be borne in small intracellular spicules which have a more or less random orientation with the cells (Fig. 12). The outer surface is bounded by a dense layer, but no internal structure is evident within the spicule proper. The pigment does not seem to be borne in any special vacuole or organelle although the cytoplasm between the clusters of spicules is less dense and lacks the granular nature of the rest of the general background cytoplasm. The pigment is dense enough to form a brown layer visible even in 1 μ sections. With polarized light it shows a strong light-blue birefringence in Epon sections. Our micrographs provide no further information as to the origin or development of these brown pigment spicules.

Freshly captured specimens produced a steady glow of blue light which was in a highly directional beam aimed anterioventrally. In freshly dissected photophores the posterior cup iridophores reflected blue to blue-green light normal to the platelets of the iridophore. The irregular iridophores have a "frosted-silver" appearance when viewed at normal angles but at oblique angles are translucent. When illuminated normal to the platelet surface the anterior cap iridophores reflect yellow light and transmit blue and red light. It was extremely difficult to make reflectance observations on the transparent iridophores of the lens because in the intact photophore the reflection of the other iridophores caused confusion. When the lenses were dissected off the photophores they reflected yellow light and transmitted blue at angles normal to the platelet axis.

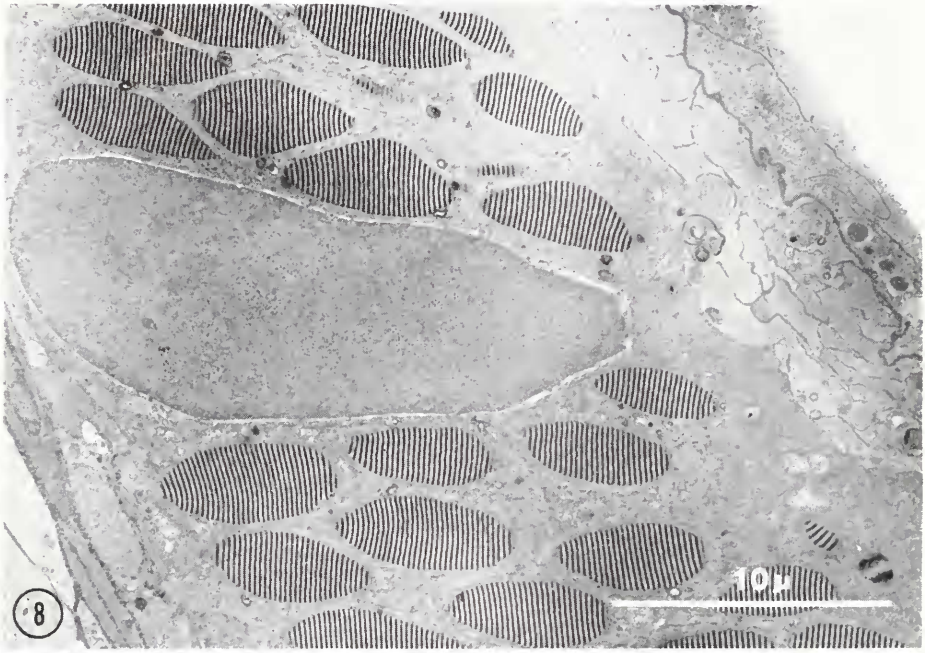
Discussion

The major question to be considered in this paper is the possible function and significance of the various types of iridophores found in the photophore. Denton

Figure 5. Iridophores of the "plug" region of the axial cone. Note the iridophores are separated by a dense floccular material and the iridosomes can be convoluted.

Figure 6. Irregular iridophores in the layer outside the axial cone. The platelets tend to run in parallel arrays but vary in spacing and thickness and are covered with protrusions.

Figure 7. Higher magnification of the irregular iridophore platelets. Microtubules (mt) can occasionally be seen in association with the forming ends of the platelet. The protrusions on the platelet frequently appear to be hollow and seem to be randomly spaced. Fine granular material (gm) appears to be fusing to form the platelets.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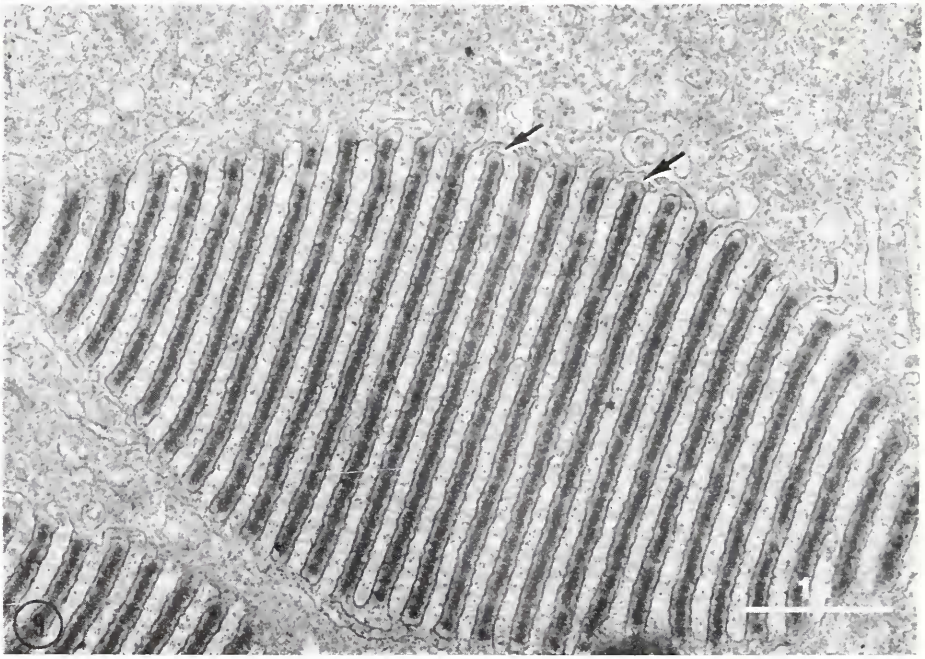
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and Land (1971) have discussed in detail the mechanism of reflectance of iridophores in fish and cephalopods. Huxley (1968) has provided a theoretical basis for the optical behavior of such reflectors, and Land (1972) has summarized both the physical and biological aspects of iridophore reflectance. In both ideal and non-ideal multilayer systems the first-order reflectance peak occurs at $\lambda_{max} = 2\ (n_a d_a + n_b d_b)$ where n_a and n_b are respectively the reflective indices of the optically light and dense layers in the stack, and where d_a and d_b are respectively the thicknesses of these two layers (Land, 1972). We are somewhat hampered in the interpretation of the function of the iridophores because we do not have a direct measurement of the refractive index of either the platelets (optically dense layers) or the spaces (optically light layers) between them. However, Denton and Land (1971) have published values of 1.56 for the refractive indexes of the platelets from the "eyelid" of *Loligo forbesi* and *Sepia elegans*. Following Land (1972) we have assumed a refractive index of 1.33 for the optically light layers. Unfortunately the calculated λ_{max} values based on these indexes and measurements of thicknesses from photomicrographs do not agree with observed reflections from freshly dissected photophores. The most likely source of error is the measurements of the spaces between platelets which may suffer considerable shrinkage during fixation and embedding. Indeed by ignoring the measurements of the spaces and assuming the stacks represent ideal multilayers based on the mean thickness of the platelets, rather close agreement is achieved between observed and calculated reflectance in several cases.

Calculations of reflectance for the regular iridophores in the posterior cup give a λ_{max} of 43 nm which agrees with the subjectively determined blue reflection of freshly dissected iridophores. The orientation of these reflectors suggest that they function in redirecting light that would otherwise be lost. By selectively reflecting blue light these iridophores act as a color filter. Since another color filter is probably present in the anterior cap (see below) this system may seem redundant. However by reflecting only blue light, a light trap (the pigment screen surrounding the posterior cup) is provided for light reflected by the anterior cap iridophores.

Calculations of reflectance for the anterior cap iridophores gives a λ_{max} of 53 nm which agrees fairly well with the subjectively observed yellow reflection. Thus these iridophores will act as a color filter by passing blue light while reflecting probably yellow-green light back into the photophore.

The iridophores of the "plug" region of the anterior cap have somewhat different reflective characteristics. While measurements have not been made on the platelets, observations on fresh photophores indicate they reflect red light. It seems unlikely, however, that the photocytes produce light at these frequencies. These iridophores are attached to muscles which apparently have their origin out-

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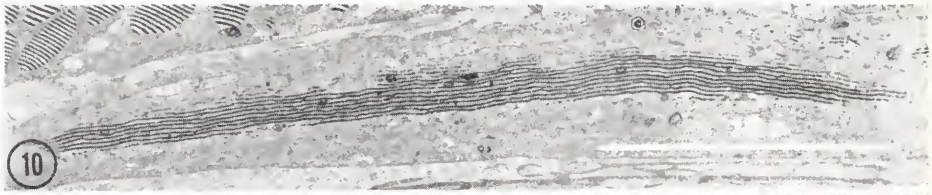
FIGURE 8. Transparent iridophores of the lens. These iridophores contain many iridosomes, each of which has its platelets aligned with platelets in other iridosomes. Thus the whole lens is precisely oriented. The surface of the lens is covered with muscle processes and connective tissue.

FIGURE 9. Higher magnification of a single transparent iridosome. Compare the interplatelet spacing with that of the regular iridosomes. Note that some of the platelets are continuous with the cytoplasm but that others end with a membrane cap (arrows).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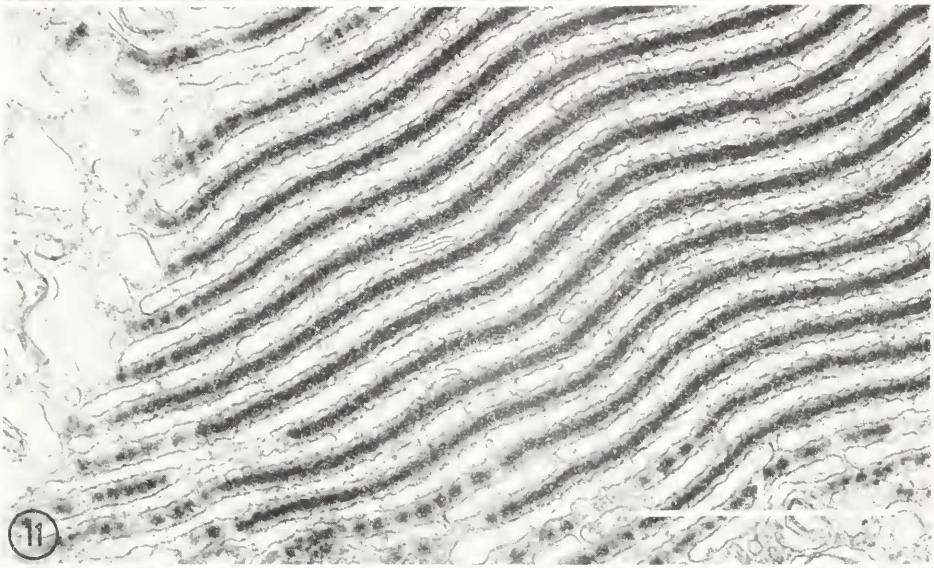
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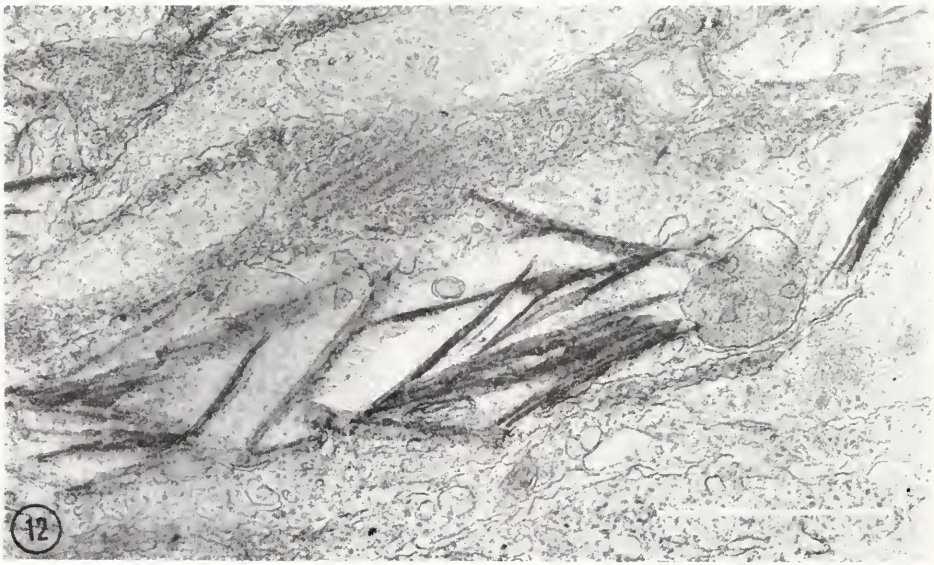
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side the photophore proper. Tensional forces on the periphery of the iridophore from contraction of the muscles may result in reducing the spacing between the platelets thereby altering their transmittance and reflectance characteristics. This may provide a mechanism for regulating the intensity of emitted light perhaps by shifting the reflectance band into and out of the blue region.

The irregular iridophores also offer some interesting ground for speculation. Since the spacing is so irregular and since platelets are covered with protrusions and knobs, they could not possibly function as ideal quarter λ stacks. Their structure suggests they may diffusely reflect light back into the region of the axial cone. In this way light would be redirected but not directionalized as would be the case with the regular iridophores. This assumption agrees well with observations on freshly dissected photophores.

The lens iridophores are very different from the others. All stacks have similar alignment, and they have thin platelets and very thick spaces. These are clearly non-ideal multilayers. If the optical thicknesses of the platelets and spaces have a ratio of 1 : 3 (this assumes approximately the same degree of shrinkage as in the anterior cap and posterior cup iridophores), the calculated reflectance peak is at 54 nm or approximately the same as the anterior cap iridophores. This peak agrees reasonably well with the yellow reflection these iridophores seem to give in fresh photophores. Presumably only blue light reaches the lens iridophores because of the filtering effects of the anterior cap iridophores. Blue light arriving at normal incidence to the lens iridosomes will pass through and out of the photophore. Blue light arriving at oblique angles may be reflected back into the photophores, however the transmittance and reflectance characteristics of these iridosomes require more careful scrutiny. Certainly the precise alignment of the iridosomes suggests they may act to collimate the light.

It would seem, therefore, that the photophore of this midwater squid is a complex organ potentially capable of producing light of controlled wave length in a highly directional beam whose intensity can be regulated. The individual organs are all oriented in the same plane so that a beam of light could be directed vertically downward from the eyes. These organs, therefore, seem to meet all the requirements necessary for ventral countershading.

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FIGURE 10. Wide spaced iridophore. This type of iridophore tends to be wavy and the platelets loosely parallel each other. The iridosome occupies a relatively small volume of the cell. This type of iridophore can be found in the skin or irregularly placed on the photophore.

FIGURE 11. Higher magnification of the wide spaced iridophore. Except for the spacing the iridosome seems similar to the regular iridophore.

FIGURE 12. Intracellular pigment spicules of the brown pigment layer surrounding the proximal posterior cup. The individual spicules more or less parallel the long axis of the tissue layer containing them.

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Summary

The iridophores of one type of photophore of the deep sea squid, *Pterygioteuthis microlampas* were examined with the electron microscope and four different types were found. Three of these types have not been previously described. The regular iridophores of the posterior cup appear to be one-fourth wave length reflectors and redirect the light produced by the photogenic tissue outward. The regular iridophores of the anterior cap have a different spacing and platelet thickness so they apparently pass blue light. The irregular iridophores form a cone around the photogenic tissue and probably randomly reflect light back into the photogenic tissue. The iridophores of the lens have many precisely aligned iridosomes with platelet spacing and thickness so that they appear to collimate light passing through them. It appears that these three types of iridophores reflect, transmit and collimate the light produced in the photophore to match the background illumination hence making an efficient countershading mechanism. A fourth type of iridophore, the wide spaced iridophore, is rarely encountered and probably does not have a significant role in light attenuation in the photophore.

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